In vitro shoot growth and root formation enhancement of *Moringa oleifera* Linn. on DKW medium containing cytokinins and auxins

Cite as: AIP Conference Proceedings **2606**, 040008 (2023); https://doi.org/10.1063/5.0118345
Published Online: 04 January 2023

Rudiyanto, Agus Purwito, Darda Efendi, et al.

In vitro Shoot Growth and Root Formation Enhancement of *Moringa oleifera* Linn. on DKW Medium Containing Cytokinins and Auxins

Rudiyanto[1, 2, a)], Agus Purwito[2], Darda Efendi[2] and Tri Muji Ermayanti[1]

[1]*Research Center for Biotechnology, National Research and Innovation Agency (BRIN), Jl. Raya Bogor Km. 46, Cibinong, 16911, West Java, Indonesia.*
[2]*Departement of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Jl. Meranti, Dramaga, Bogor, 16680, West Java, Indonesia.*

[a)] Corresponding author: *rudidinasty@yahoo.com*

Abstract. Moringa (*Moringa oleifera* Linn.) is a popular plant as a functional food crop. In micropropagation, modifying the medium culture is critical to determine the best nutrient composition for plantlet production. The antecedent research showed that the basic DKW (Driver and Kuniyaki Walnut) medium was the best compared to MS (Murashige and Skoog), WPM (Woody Plant Medium), NN (Nitsch and Nitsch), and B5 (Gamborg) media. This experiment aimed to enhance the growth of shoot culture and its root formation on a basic DKW medium supplemented with cytokinins and auxins. Two experiments were conducted. The first experiment was designed to obtain the best medium for shoot growth by adding cytokinin BAP, Kinetin, 2-IP, and Zeatin. The use of IAA (indole-3-acetic acid), NAA (1-naphthalene acetic acid), and IBA (3-indolebutyric acid) to enhance root formation was set for the second experiment. A completely randomized design with two elements was utilized for the experiment. The variables tried were: Plant Growth Regulators (PGR): BAP, Kinetin, 2-IP, and Zeatin with 0.0, 0.5, 1.0, and 2.0 mg/l for shoot development and IAA, NAA, and IBA at a similar concentration for root arrangement. Every treatment comprised of 12 recreates. The factors noticed were shoot stature, shoot numbers, petiole numbers, root numbers, and root length noticed one time per week. Following a month and a half after culture, plantlet performance were recorded. The best shoot stature, shoot numbers, and petiole numbers were created on a medium enhanced with 1 mg/l Kinetin and significantly unique with others. The most significant roots and root length were created at 1.0 and 2.0 mg/l IAA.

INTRODUCTION

Moringa oleifera Linn. is currently utilized as a functional food and is generally developed universally because it has a few advantages for human wellbeing. This plant species is local to Middle Eastern nations: India, Bangladesh, Pakistan, and others [1,2,3]. This plant has become naturalized in the tropics and subtropics districts with proceeded development [4].

Moringa oleifera is also famous as an alternative source for hidden hunger health issues, especially preventing and alleviating malnutrition [5]. Its seeds and leaves are commonly eaten fresh, cooked, or powdered. They contain numerous essential synthetic substances like unsaturated fats, β-carotene, tocopherols, and phenolic compounds, beneficial for nutrients and health-promoting compounds. The natural products are stringy and customarily used to forestall colon disease and treat stomach related issues. Blossom extracts improve the taste and shade of dishes in culinary arrangements [5,6,7].

Conventional propagation of *M. oleifera* commonly done by generative method uses seeds or vegetatively by cuttings. This method has some disadvantages, such as limited explants for cuttings, susceptibility to microbial diseases, and seed viability. Micropropagation through *in vitro* culture offers some advantages, such as being stable, free of pests and diseases, and producing relatively quickly and producing uniform seeds. Micropropagation is impacted by many variables, like culture medium, plant development controllers, and plant species genotype [8].

Proceedings of the 8th International Symposium of Innovative Bioproduction Indonesia on Biotechnology and Bioengineering 2021
AIP Conf. Proc. 2606, 040008-1–040008-15; https://doi.org/10.1063/5.0118345
Published by AIP Publishing. 978-0-7354-4305-1/$30.00

One of the fundamental variables in plant tissue culture, particularly in the multiplication stage, is adding plant development controllers into the way of medium, like cytokinin and auxin. It is notable that cytokinins assume different parts in plant advancement, for example, the exercises of specific catalysts and advancing cell division and cell extension in plant protein combination excitement [9]. Auxin is a focal part that directs root improvement, particularly primer and horizontal roots and root hairs [10].

Principally, cytokinins play a critical part in plant improvement, such as advancing cell division and extension and the guidelines for shoot development and duplication. BAP improves shoot arrangement in the interim and can set sidelong buds free from lethargy. In *C. orivilianum* refined, 26.6 µM of BAP could upgrade the most elevated shoot number while significantly reducing shoot length [11].

Auxin is the most generally concentrated on phytohormone. It is associated with plant development and advancement, for example, organogenesis, embryogenesis, tissue designing, and tropism [12]. Indole-3-acidic-acid (IAA) is the most bountiful typical of auxin found broadly across the plants, trailed by indole-3-butyric-acid (IBA). IAA has been displayed to control different phases of root advancement and root framework design, though IBA advances lateral root improvement [12]. In addition, engineered types of auxins, like 2,4-D (2,4-dichlorophenoxyacetic acid) and NAA (1-naphthaleneacetic acid), are regularly utilized in plant tissue culture examination to control development. Auxin goes about as a focal part that directs root improvement, particularly the arrangement of sidelong and primer roots and root hairs [10].

In our past outcomes, the ideal development of *M. oleifera* refined was accomplished on DKW medium, which delivered the most noteworthy shoot stature (13,97 cm), petioles (12,33), and the root numbers (8.33) [13]. BAP at 4.44 µM was ideal for delivering the most significant number of shoots per explants. In the meantime, in 2.85 µM IAA and 4.92 µM IBA treatment acquired proficiently in vitro establishing of individual shoot culture [14]. BAP at 2.5 µM created a normal of 4.6 axillary shoots per explant following fourteen days [15]. This experiment was meant to improve the shoot culture of *M. oleifera* and its root development on DKW medium enhanced with cytokinins and auxins.

MATERIALS AND METHODS

Materials

Materials utilized in this experiment were plantlets of *M. oleifera* at about a month and a half of refined on DKW medium. In this examination, 1.5-2.0 cm long of internodes of *M. oleifera*, with 2-3 leaves, were refined by the medium treatment tried. The Plant Growth Regulators (PGR) utilized were BAP, Kinetin, 2-IP, and Zeatin at 0.0, 0.5, 1.0, and 2.0 mg/l for shoot development advancement (First experiment) and IAA, NAA, and IBA at a similar concentration for root arrangement (Second experiment). The media were set utilizing 3 g/l of Gelzan (TM Caisson labs). Medium pH was changed by 5.8 and afterwards sterilized with an autoclave at 121°C at 15 psi for 20 min. Cultures were brooded at 26 ± 2°C, with 500-900 lux of photoperiod light.

Experimental Design and Data Analysis

The experimental plan utilized was Complete Random Design factorial. In the primary analysis, the principal factor was the kind of cytokinins: BAP, Kinetin, 2-IP, and Zeatin; the next element was the various fixations at levels 0.0, 0.5, 1.0, and 2.0 mg/l for the shoot development treatment. In the subsequent test, the primary element was the kind of auxin: IAA, NAA, and IBA, while the subsequent variable was the concentration of 0.0, 0.5, 1.0, and 2.0 mg/l for the root development treatment. Analysis of Variance (ANOVA) was utilized for quantitative information examined to decide the impact between treatment. The essentially various factors were tried utilizing DMRT (Duncan's Multiple Range Test) at α 1 and 5% levels utilizing open-source programming (DSAASTAT V.1.1). Every treatment comprised of 12 recreates. The factors noticed were shoots tallness, shoot numbers, petiole numbers, root numbers, and root length noticed each week until about a month and a half of culture. At about a month and a half of culture, plantlet performance were recorded.

RESULTS AND DISCUSSION

Effect of Cytokinin on Shoot Growth

The impact of cytokinins on plant micropropagation can can vary based on the variety of plants, the age of explants, and the kind of basic culture medium. Some past research showed that ideal convergences of adenine-type cytokinins are vital for tissue culture and in vitro multiplication [9]. The development of *M. oleifera* shoot refined on DKW medium enhanced with BAP, Kinetin, 2-IP, and Zeatin at 0.0 (control treatment), 0.5, 1.0, and 2.0 mg/l is displayed in Fig. 1. In the BAP medium, shoot stature development started a week after culture. The shoot stature kept on expanding until about a month and a half after culture. In DKW medium containing 0.0 and 0,5 mg/l BAP, the ideal shoot stature was reached at 2 a month of culture. The expansion of BAP at 2.0 mg/l slow the shoot stature (Fig. 1a). Kinetin gave a vast expansion in the shoot tallness at 2 a month and a half after culture. Moreover, the expansion of Kinetin at 1.0 mg/l created the most elevated shoots (Fig. 1b).

Figure 1c shows that shoots development of *M. oleifera* started fourteen days after culture on DKW medium containing 2-iP. The increment of shoot stature went on until about a month and a half after culture. Stronghold with 1.0 mg/l 2-iP gave the most noteworthy shoot of *M. oliefera*. At 0; 0.5 and 2.0 mg/l 2-iP gave comparable development. This outcome is compared with the expansion of Zeatin. The development of shoot tallness was likewise ideal at two weeks until a month and a half after culture. Without Zeatin and with the expansion of 0.5, 1.0 and 2.0 mg/l gave no critical distinction on the shoot tallness (Fig. 1d).

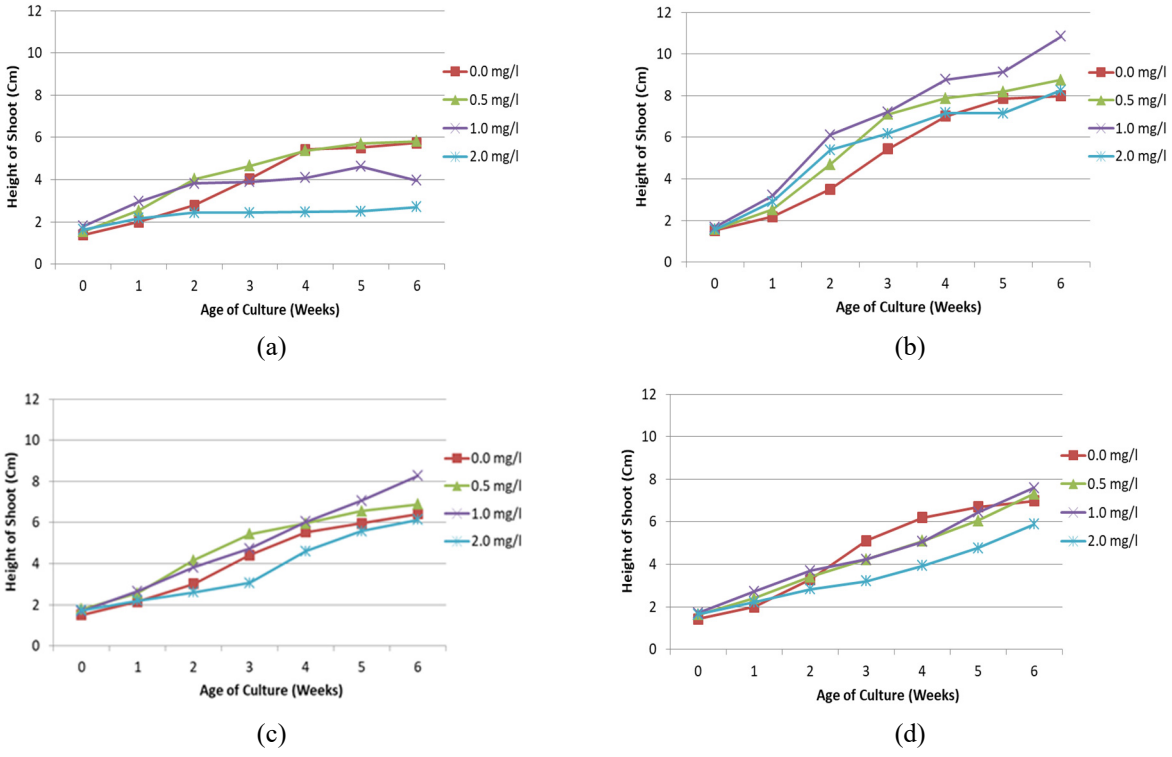

FIGURE 1. Shoot stature of *M. oleifera* grown on DKW medium supplemented with 0.0; 0.5; 1.0 and 2.0 mg/l BAP (a), Kinetin (b), 2-iP (c), and Zeatin (d) refined for 0-6 weeks

Figure 2 shows the expanding shoot numbers of *M. oleifera* refined in DKW medium enhanced with various cytokinins. The addition of BAP expanded the quantity of shoots at 2 and 3 weeks after culture. The ideal development was found in the medium with 0,5 mg/l BAP, which created the most significant number of shoots at 3 weeks until a month and a half after culture. The development of the number of shoots in DKW containing 0.0, 1.0, and 2.0 mg/l BAP on 2 weeks until a month and a half on culture was not significant (Fig. 2a). A low fixation (0.5 mg/l Kinetin and BAP) was added to MS medium expanded horizontal shoots in *R. akane*. Micropropagation of this

plant species has not been generally revealed. However, in the same genus, *R. cordifolia*, it requires BAP with a higher concentration (1 mg/l) to obtain an optimal number of shoots [16][17].

In the medium enhanced with Kinetin, the shoot numbers of *M. oleifera* shifted, relying upon the various fixations given. The number of shoots expanded after fourteen days. The most significant shoot numbers was produced in the medium with 1.0 mg/l Kinetin, trailed by 0.5, 2.0, and 0.0 mg/l Kinetin, separately, at about a month and a half after culture (Fig. 2b). In DKW medium containing 2-iP, the expanding number of the shoot was lower than Kinetin or BAP. The most significant number of shoots was produced in the medium with 0.5 mg/l 2-iP at 3 weeks until a month and a half after culture (Fig. 2c). Zeatin did not altogether upgrade shoot numbers. The development was delayed from 0 to about a month and a half after culture (Fig. 2d).

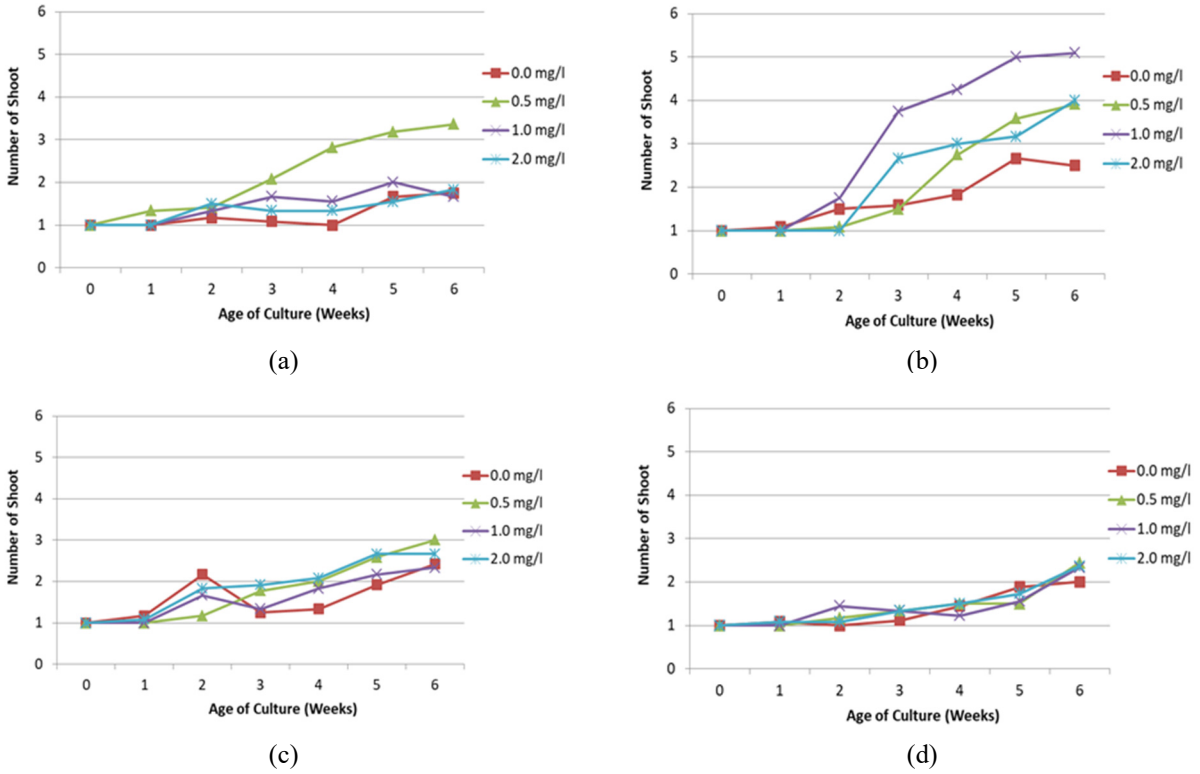

FIGURE 2. Shoot numbers of *M. oleifera* grown on DKW medium supplemented with 0.0; 0.5; 1.0 and 2.0 mg/l BAP (a), Kinetin (b), 2-iP (c), and Zeatin (d) refined for 0-6 weeks

The expanding petiole numbers in *M. oleifera* refined on the medium containing BAP, Kinetin, 2-iP, and Zeatin at 0 to about a month and a half after the culture is displayed in Fig. 3. BAP expanded the petiole numbers at 2-5 weeks. At about a month and a half, the most extensive petiole numbers was found in media with 0.5 mg/l BAP (Fig. 3a). [18]; [17] stated that when the availability of cytokinins in the culture medium was too high, cell division in the cultured tissue would be hampered. However, when the tissue is subcultured on a medium with optimal cytokinin content, cell division can take place more quickly, and plantlet growth can take place optimally. Cytokinin additionally impacted the development of *M. oleifera* shoot culture. Kinetin gave the number of petioles differed in every concentration. The number of petioles started to build after fourteen days. The most significant petiole numbers was found in the medium containing Kinetin at 1.0 mg/l. The ideal petiole numbers expanded at 2 weeks until a month and a half after culture (Fig. 3b). The average quantity of petioles on DKW medium containing 2-iP at 0.0, 0,5. 1.0 and 2.0 mg/l at 2 a month and a half were not significant. Few petioles were seen in the medium without adding 2-iP (Fig. 3c). Also, the expansion of Zeatin didn't upgrade the petiole numbers at 2 weeks until a month and a half of culture (Fig. 3d).

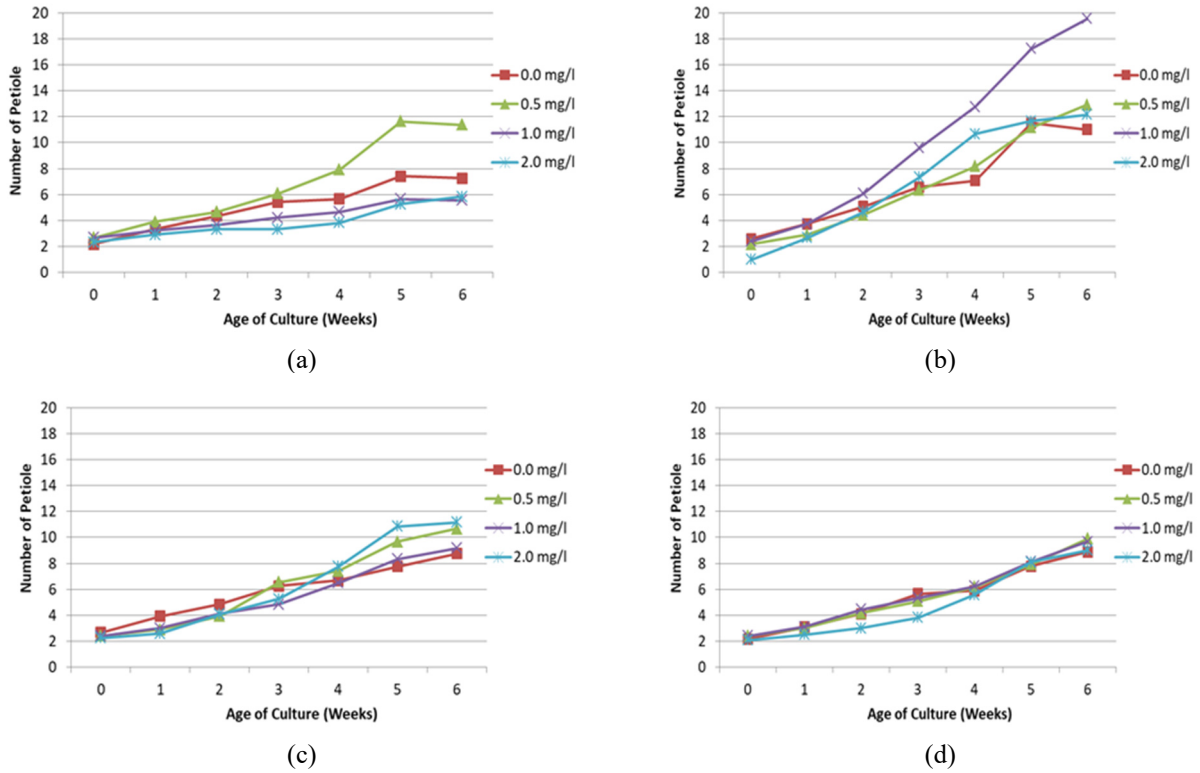

FIGURE 3. Petiole numbers of *M. oleifera* grown on DKW medium supplemented with 0.0; 0.5; 1.0 and 2.0 mg/l BAP (a), Kinetin (b), 2-iP (c), and Zeatin (d) refined for 0-6 weeks

Figure 4 shows the quantity of roots of *M. oleifera* refined on DKW media containing four kinds of cytokinins (BAP, Kinetin, 2-iP, and Zeatin). Roots started to frame after fourteen days in the medium containing BAP. The average root numbers on the control medium (without BAP) was the most elevated. Few roots were seen in the medium with the expansion of 0.5 mg/l BAP. Furthermore, there is no roots development in 1.0 and 2.0 mg/l BAP (Fig. 4a). Fig. 4b shows that the biggest number of roots was seen in the medium with no Kinetin, where the expanding number of roots started fourteen days after culture. Few roots were shaped at 0.5, 1.0, and 2.0 mg/l Kinetin (Fig. 4b). Generally, the additional cytokinins did not affect the root formation and the number of roots. However, in *M. oleifera* cultured, roots were also formed in the media with 0.5, 1.0, and 2.0 mg/l BAP and Kinetin, although only a few roots were formed. The root formation may be caused by endogenous auxin, which is generated in leaves and then transported to the basal explant to initiate roots. [19]; [20] expressed that cytokinins assume a significant part in numerous parts of plant development and advancement, including the arrangement of root and shoot meristems and vascular tissue improvement. Cytokinins additionally balance root lengthening and parallel root number. In the medium containing 2-iP, roots began to frame after fourteen days. In the control medium (without 2-iP), the ideal root numbers expanded from 2 weeks to about a month and a half. The quantity of roots was higher than in the medium with 2-iP at 0.5, 1.0 and 2.0 mg/l (Fig. 4c). Zeatin at 0.5, 1.0 and 2.0 mg/l gave no roots arrangement. The ideal number of roots expanded from 2 weeks until a month and a half after culture in the medium without Zeatin (Fig. 4d).

Variance Analysis or ANOVA on shoot stature, shoot numbers, petiole numbers, and quantity of roots of *M. oleifera* on DKW medium enhanced with four kinds of cytokinins are displayed in Table 1. The outcomes show that cytokinins had exceptionally critical impacts on shoot stature, the shoot numbers, and the petiole numbers, and a significant impact on the quantity of roots. In comparison, in concentration treatment likewise has an exceptionally critical impact on shoot stature, shoot numbers, petiole numbers, and root numbers. There was an association between cytokinins and concentration on the quantity of shoots and petioles. Nonetheless, there were no associations between the quantity of roots and shoot stature (Table 1).

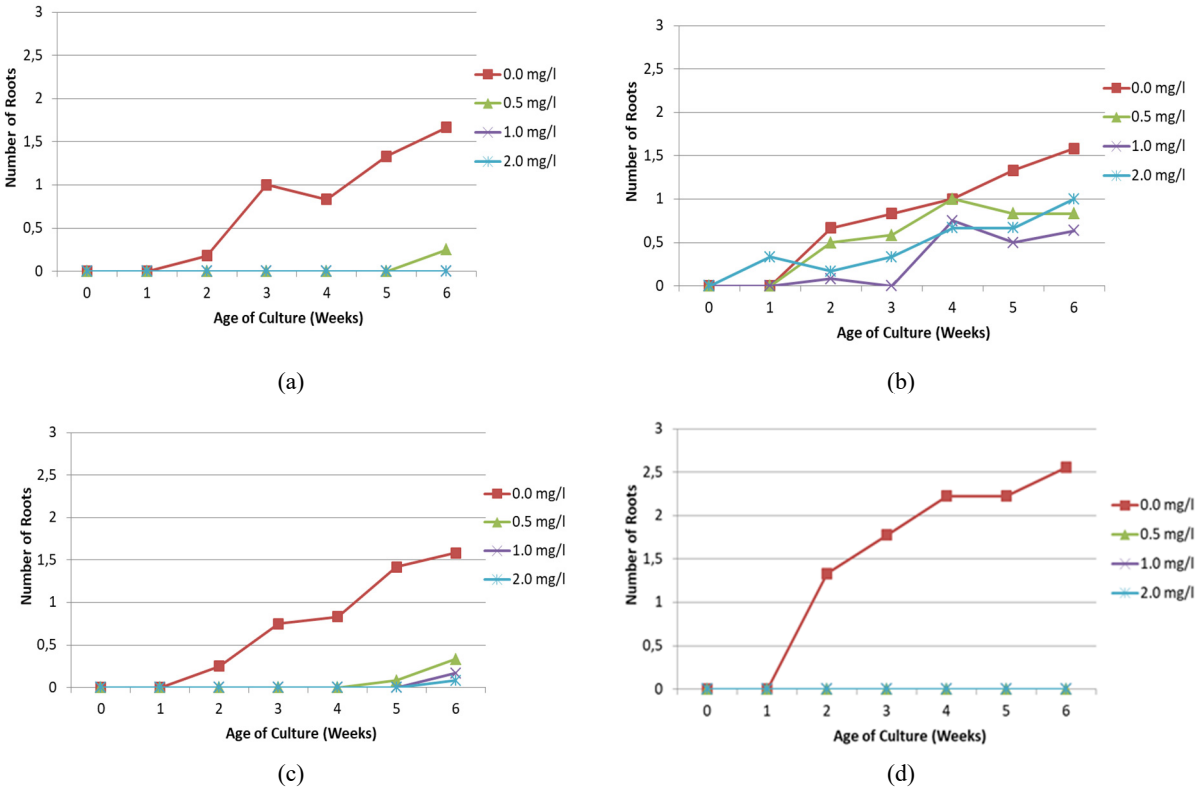

FIGURE 4. Root numbers of *M. oleifera* grown on DKW medium supplemented with 0.0; 0.5; 1.0 and 2.0 mg/l BAP (a), Kinetin (b), 2-iP (c), and Zeatin (d) refined for 0-6 weeks

TABLE 1. Variance Analysis or ANOVA on shoot stature, shoot numbers, petiole numbers, and root numbers of *M. oleifera* cultured on DKW medium supplemented with BAP, Kinetin, 2-iP and Zeatin six weeks after culture

No	Variables	F Value and Significance			CV (%)
		Cytokinins	Concentrations	Cytokinins Vs Concentrations	
1.	Shoot Height	25.79^{**}	5.18^{**}	1.76^{ts}	31.38
2.	Number of Shoots	23.59^{**}	5.82^{**}	3.45^{**}	36.13
3.	Number of Petioles	26.67^{**}	4.08^{**}	6.02^{**}	29.94
4.	Number of Roots	3.87^{*}	29.06^{**}	1.38^{ts}	134.00

Note * : significance at α: 5%; **: very significance at α 1%; ns : not significance

Table 2 shows the development of *M. oleifera* refined on DKW medium containing BAP, Kinetin, 2-iP, and Zeatin at 0.0, 0.5, 1.0, and 2.0 mg/l at about a month and a half after culture. The most noteworthy shoot was found in the medium supplemented with 1.0 mg/l Kinetin, which is significantly different from other treatments with different levels and cytokinins. The most minimal shoot was found in the medium with BAP at 1.0 and 2.0 mg/l (Table 2). In accordance with that, [9] revealed that higher addition of BAP (2 mg/l) repressed the shoot expansion of *Amygdalus communis*, diminished multiplication rate, length of small shoot, and expanded callus and mini shoot. Our observing showed that the most significant shoot numbers and petiole numbers of Moringa refined on the medium using 1.0 mg/l Kinetin was significantly different from others. The expansion of 1.0 mg/l Kinetin created the most significant shoot numbers and quantities of petioles. Conversely, a low shoot numbers and petioles was found in the media containing 1.0 and 2.0 mg/l of BAP and Zeatin (Table 2). Kinetin is an essential plant hormone.

Stimulating cell division regulates leaf primordia number, shoot meristem size, and leaf and shoots growth. Kinetin can also stimulate mediate axillary bud release from apical dominance [21]. Kinds of cytokinins effectively affect the development of Dahlia shoots in tissue culture. Cytokinin 2-iP (0.1-2.0 mg/l) gave a slow reaction to the development of Dahlia shoots contrasted with different treatment. Cytokinin 0.1-2.0 mg/l BAP couldn't invigorate shoot development [22]. Conversely, our finding showed that the largest number of roots were framed a month and a half after culture, which was found in control media (without the addition of BAP, Kinetin, 2-iP and Zeatin), while the expansion of 0.5, 1.0, and 2.0 mg/l BAP and Zeatin gave no roots arrangement (Table 2).

TABLE 2. The average value of shoot stature, shoot numbers, petiole numbers, and root numbers of *M. oleifera* cultured on DKW supplemented with BAP, Kinetin, 2-iP and Zeatin 6 weeks after culture

Cytokinin	Concentration (mg/l)	Shoot Stature (cm)	Shoot Numbers	Petiole Numbers	Root Numbers
BAP	0.0	5.80 ± 0.74^{efg}	1.78 ± 0.36^{ef}	7.33 ± 0.71^{ef}	1.67 ± 0.24^{de}
	0.5	5.58 ± 0.67^{fg}	3.22 ± 0.36^{bcd}	11.44 ± 1.25^{bcd}	0.00 ± 0.00^{e}
	1.0	3.97 ± 0.34^{gh}	1.67 ± 0.33^{f}	5.56 ± 1.03^{f}	0.00 ± 0.00^{e}
	2.0	2.73 ± 0.16^{h}	1.89 ± 0.26^{ef}	5.78 ± 0.64^{f}	0.00 ± 0.00^{e}
Kinetin	0.0	7.96 ± 0.46^{bcd}	2.56 ± 0.18^{def}	11.00 ± 0.80^{bcd}	**1.56 ± 0.50^{ab}**
	0.5	8.69 ± 0.56^{b}	3.89 ± 0.31^{bc}	12.67 ± 0.67^{b}	0.89 ± 0.35^{bcd}
	1.0	**10.93 ± 0.60^{a}**	**5.11 ± 0.26^{a}**	**19.22 ± 1.40^{a}**	0.67 ± 0.33^{cde}
	2.0	8.25 ± 0.80^{bc}	4.00 ± 0.50^{b}	12.11 ± 1.39^{bc}	1.00 ± 0.33^{bc}
2-iP	0.0	6.61 ± 1.00^{cdef}	2.44 ± 0.29^{def}	8.78 ± 0.85^{de}	**1.56 ± 0.44^{ab}**
	0.5	6.76 ± 0.74^{bcdef}	3.00 ± 0.41^{cd}	10.33 ± 0.85^{bcd}	0.33 ± 0.24^{cde}
	1.0	8.24 ± 0.64^{bc}	2.33 ± 0.29^{def}	9.22 ± 0.91^{de}	0.00 ± 0.00^{e}
	2.0	6.16 ± 0.47^{def}	2.67 ± 0.41^{de}	11.22 ± 1.16^{bcd}	0.11 ± 0.11^{de}
Zeatin	0.0	6.98 ± 0.88^{bcdef}	2.00 ± 0.37^{ef}	8.89 ± 0.84^{de}	**2.22 ± 0.57^{a}**
	0.5	7.31 ± 0.92^{bcdef}	2.44 ± 0.29^{def}	9.89 ± 1.23^{bcde}	0.00 ± 0.00^{e}
	1.0	7.59 ± 1.07^{bcde}	2.33 ± 0.24^{def}	9.67 ± 0.83^{cde}	0.00 ± 0.00^{e}
	2.0	5.81 ± 0.77^{efg}	2.33 ± 0.24^{def}	9.11 ± 1.16^{de}	0.00 ± 0.00^{e}

Note: The numbers followed by the same letter in the same column are not significantly different according to Duncan's multiple range test at $\alpha = 5\%$

The presentation of *M. oleifera* refined on DKW containing various of cytokinins is displayed in Fig. 5. Shoots in DKW medium enhanced with BAP at 1.0 mg/l were more robust than in control and 0.5 mg/l BAP. The leaves' tone was greener with vigorous petioles and stems. At the medium containing 0.5, 1.0, and 2.0 mg/l BAP, callus was shaped, yet in the control medium, no callus was framed at the foundation of the stems (Fig. 5a). Some *in vitro* cultures only use cytokinins considering that the endogenous auxin in plants is sufficient to support root growth in plant tissue culture [23]. [24] research results; [23] stated that the highest agarwood shoots were produced at 0.5 mg/l BAP, which obtained 6.11 shoots. In contrast, when the concentrations of BAP increased, the average shoots produced decreased. High convergences of cytokinins can cause a decline in the quantity of shoots, and BAP fixations that surpass the ideal levels cause restrained shoot advancement. Figure 5b shows that Kinetin at 1.0 mg/l gave the shoot's growth superior to different media. On medium without expansion of Kinetin and 0,5 mg/l Kinetin, the stems and leaves were withered and yellowed. On the control medium, shoots did not show critical development. Calli were shaped in totally refined media at the foundation of stems (Fig. 5b). The best cytokinin is Kinetin which is usually utilized in initiating shoot creation. The adequacy of Kinetin in the movement of the catabolic chemical cytokinin oxidase plays the main job in controlling cytokinin levels. The action of Kinetin is a cytokinin corrupting protein that at last decreases the adequacy of any cytokinins in that plant contingent upon the chemical's underlying fixation [25].

Vigorous shoots of *M. oleifera* with long stems and wide leaves are displayed on DKW medium with 1.0 mg/l 2-iP. The shoots were hindered and had just few leaves on control media. The stem's bud broadened and framed a callus on the medium containing 2-iP at 0.5, 1.0, and 2.0 mg/l (Fig. 5c). DKW containing 1.0 and 2.0 mg/l Zeatin gave a more predominant impact than the control media with more vigorous stems, many leaves, and more callus. The hindered shoot was found in control media (Fig. 5d).

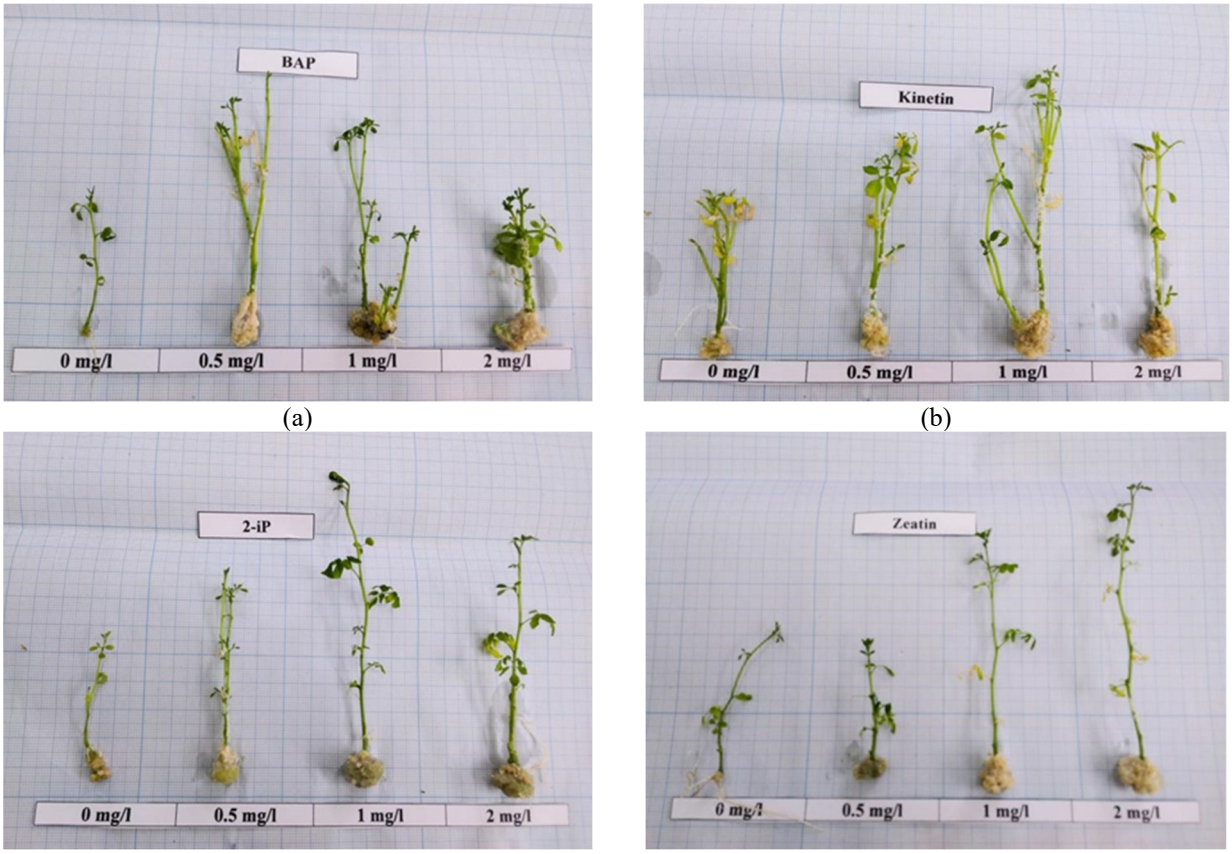

FIGURE 5. Culture performance of *M. oleifera* on DKW medium supplemented with 0.0, 0,5, 1.0 and 2.0 mg/l BAP (a), Kinetin (b), 2-iP (c), and Zeatin (d) at 6 weeks after culture.

Effect of Auxin on Root Growth

Our finding showed that *M. oleifera* shoots culture developed well on DKW medium with and without auxins IAA, IBA, and NAA. Kinds of auxins gave various statures of shoots. The expansion of 2 mg/l IAA delivered the most elevated shoot development. The most reduced shoot was gotten on the medium containing IAA at 1 mg/l. In any case, the development of shoots from the start of culture to about a month and a half of culture was not significant (Fig. 6a). The outcome showed that without IBA giving the most noteworthy shoot tallness, the briefest shoot was viewed in the medium with 2 mg/l IBA. The higher the concentration of IBA (2 mg/l) gave the least shoot tallness (Fig. 6b). Essentially, no expansion of NAA additionally brought the most elevated shoot stature. Be that as it may, the most brief shoot was found in the medium containing NAA at 1 mg/l (Fig. 6c).

The quantity of shoots of *M. oleifera* saw from 0 to about a month and a half after culture on DKW medium containing IAA, IBA, and NAA at 0.0, 0.5, 1.0 and 2.0 mg/l is displayed in Fig. 7a. The most significant number of shoots was displayed in control treatment/without IAA. Besides, the most reduced shoot numbers were viewed in the medium enhanced with 1.0 and 2.0 mg/l IAA (Fig. 7A). Medium containing 0.5 mg/l IBA gave the most extensive number of shoots. The shoot started to grow three weeks after culture. There was no considerable contrast in development between the control medium with the addition of IBA at 1.0 and 2.0 mg/l from 0 to a month and a half of culture (Fig. 7b). Conversely, the medium without NAA gave the most significant number of shoots and essentially contrasted from others. The ideal development of shoots was found at 2 weeks until a month and a half after culture (Fig. 7c)

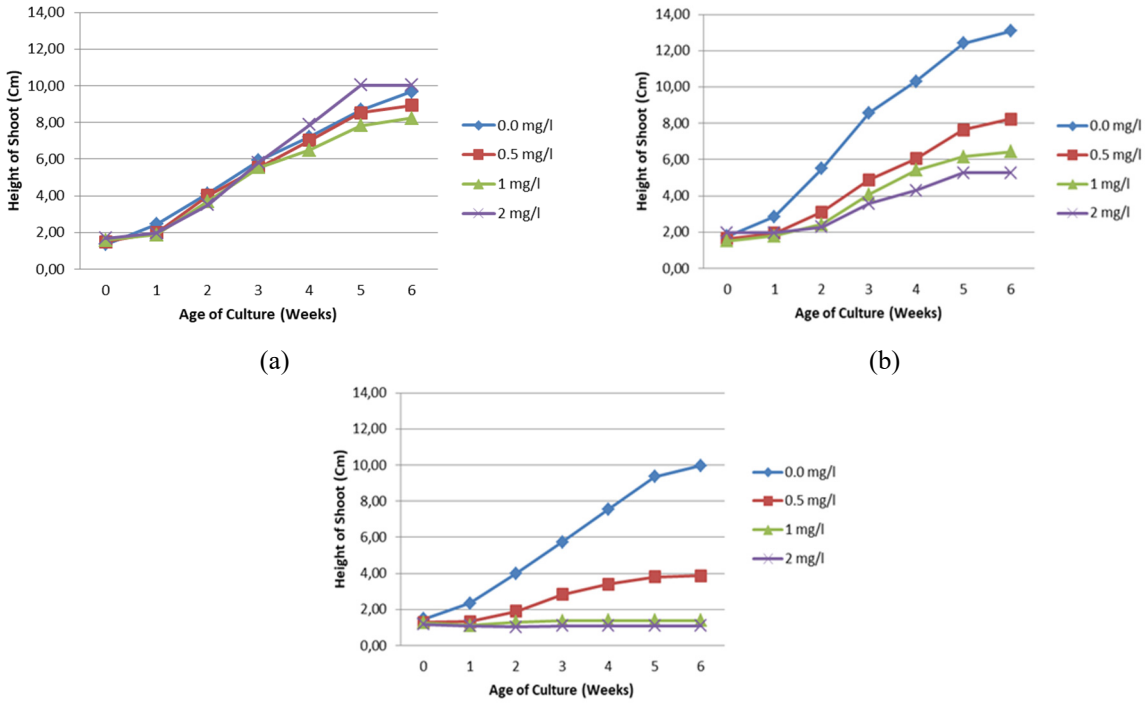

FIGURE 6. Shoot stature of *M. oleifera* grown on DKW medium supplemented with 0.0; 0.5; 1.0 and 2.0 mg/l IAA (a), IBA (b), and NAA (c) refined for 0-6 weeks

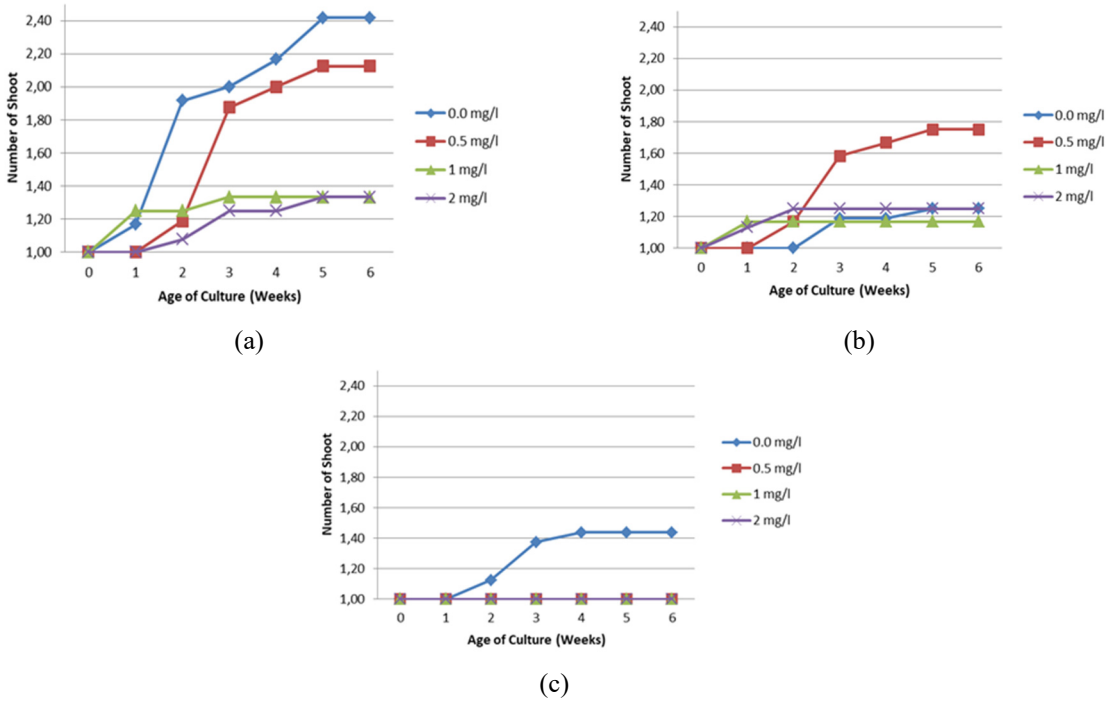

FIGURE 7. Shoot numbers of *M. oleifera* grown on DKW medium supplemented with 0.0; 0.5; 1.0 and 2.0 mg/l IAA (a), IBA (b), and NAA (c) refined for 0-6 weeks

Figure 8 indicates the expanding petiole numbers in *M. oleifera* refined on DKW enhanced with IAA, IBA and NAA from 0 to a month and a half after culture. In the medium with IAA at fourteen days after culture, the quantity of petioles expanded, and the ideal number expanded at 2-5 weeks. At a month and a half, the most significant petiole numbers was found in the control medium without IAA (Figure 8a). On medium without expansion of IBA gave the most extensive petiole numbers, and the most reduced petiole numbers was found in the medium with 2 mg/l IBA. The higher the concentration of IBA given, the lower number of petioles created (Fig. 8b). The average petiole numbers on the medium without NAA was higher at 2 weeks until a month and a half of culture, essentially different from others. Few petioles were found in the medium containing 1.0 and 2.0 mg/l NAA (Fig. 8c).

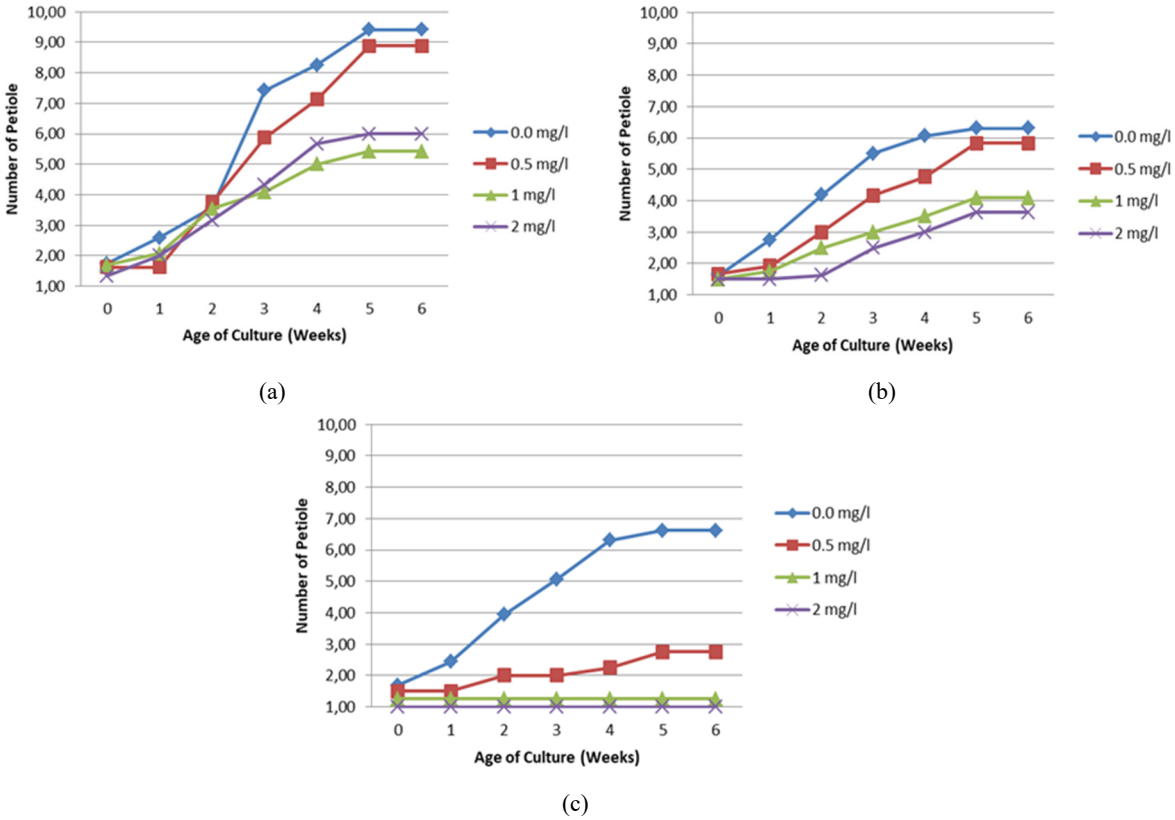

FIGURE 8. Petiole numbers of *M. oleifera* grown on DKW medium supplemented with 0.0; 0.5; 1.0 and 2.0 mg/l IAA (a), IBA (b), and NAA (c) refined for 0-6 weeks

The presence of endogenous auxin in *M. oleifera* invigorated root development. The development of roots on DKW media enhanced with IAA, IBA and NAA are introduced in Fig. 9. Numerous kinds and levels of auxins delivered established besides in the medium containing 2.0 mg/l NAA. All shoots likewise shaped roots in all control treatments or without adding auxins. The expansion of 0.5, 1.0 and 2.0 mg/l IAA improved root development at fourteen days. In the interim, roots grew a month after culture in the control medium. The most significant number of roots was gotten in the medium with IAA at 2.0 mg/l (Fig. 9a). Medium containing 2.0 mg/l IBA gave the most extensive number of roots, where the expanding number of roots started at fourteen days. A few roots were shaped in the control medium (Fig. 9b). Roots began to grow three weeks after culture in the medium containing NAA. In the control medium and the medium containing 0.5 and 1.0 mg/l NAA, the ideal number of roots expanded from 3 weeks to a month and a half. In this treatment, the quantity of roots was higher than in the medium with NAA at 2.0 mg/l (Fig. 9c). This outcome followed Gupta *et al*., (2010) [26], who detailed that auxin IAA is more appropriate for root arrangement, while NAA and IBA were more receptive to callus development.

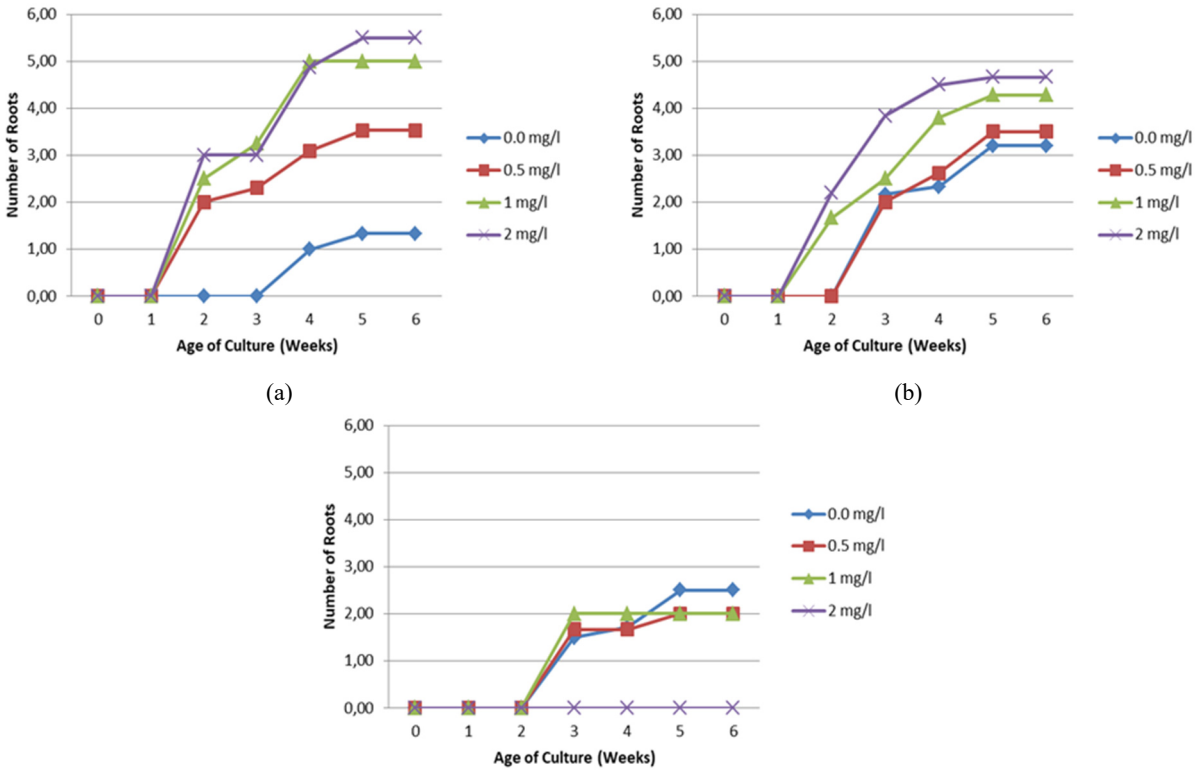

FIGURE 9. Root numbers of *M. oleifera* grown on DKW medium supplemented with 0.0; 0.5; 1.0 and 2.0 mg/l IAA (a), IBA (b), and NAA (c) refined for 0-6 weeks

The optimal auxin concentration is a crucial factor determining root growth, recorded as the number and length of roots in *M. oleifera* culture. The outcomes showed that the addition of 2.0 mg/l IAA delivered the longest roots. The ideal roots development started from 2 weeks to a month and a half after culture. The shortest root length was found in the control treatment (Fig. 10a). Conversely, the control medium gave the longest roots, and the briefest roots were found in the medium with addition 2.0 mg/l of IBA and NAA from 2 weeks to a month and a half after culture (Fig. 10B and 10C). For root induction on in vitro cultured, auxin is necessary. Masekesa *et al*., 2016 and Rantau *et al*., 2018 [25, 28] stated that adding a combination of auxin at 2 mg/l IAA and IBA on MS media showed optimal root growth, which produced 3.8 an average of roots per plantlet in guava culture. In comparison, adding IBA and IAA at 2.5 mg/l obtained 54% of rooted formation.

Table 3 shows variance analysis or ANOVA in factors: shoot tallness, shoot numbers, petiole numbers, root numbers, and roots length of *M. oleifera* on DKW medium enhanced with three kinds of auxins. The outcomes showed that various kinds of auxins had a profoundly massive impact on the factors of shoot stature, shoot numbers, petiole numbers, root numbers, and roots length. This finding follows concentration treatment, which significantly impacted factors: shoot stature, shoot numbers, petiole numbers, root numbers, and roots length (Table 3). Table 3 additionally showed interaction among auxins and concentration on factors: shoots tallness, the quantity of shoots, quantities of roots, and roots length, and there are no associations on petiole numbers.

The average shoot tallness, petiole numbers, shoot numbers, root numbers, and roots length of *M. oleifera* refined on DKW medium supplemented with IAA, IBA, and NAA at 0.0, 0.5, 1.0, and 2.0 mg/l at a month and a half are displayed in Table 4. The most elevated shoot was found in the control medium and altogether not the same as others. The last shoot was found in the medium with addition 1.0 and 2.0 mg/l NAA (Table 4). The outcomes showed that the most significant petiole numbers and shoots were delivered in the control medium and 0.5 mg/l IAA, which was fundamentally different from others. In the meantime, the most minimal shoot numbers and petioles was found in medium with 1.0 and 2.0 mg/l NAA (Table 4).

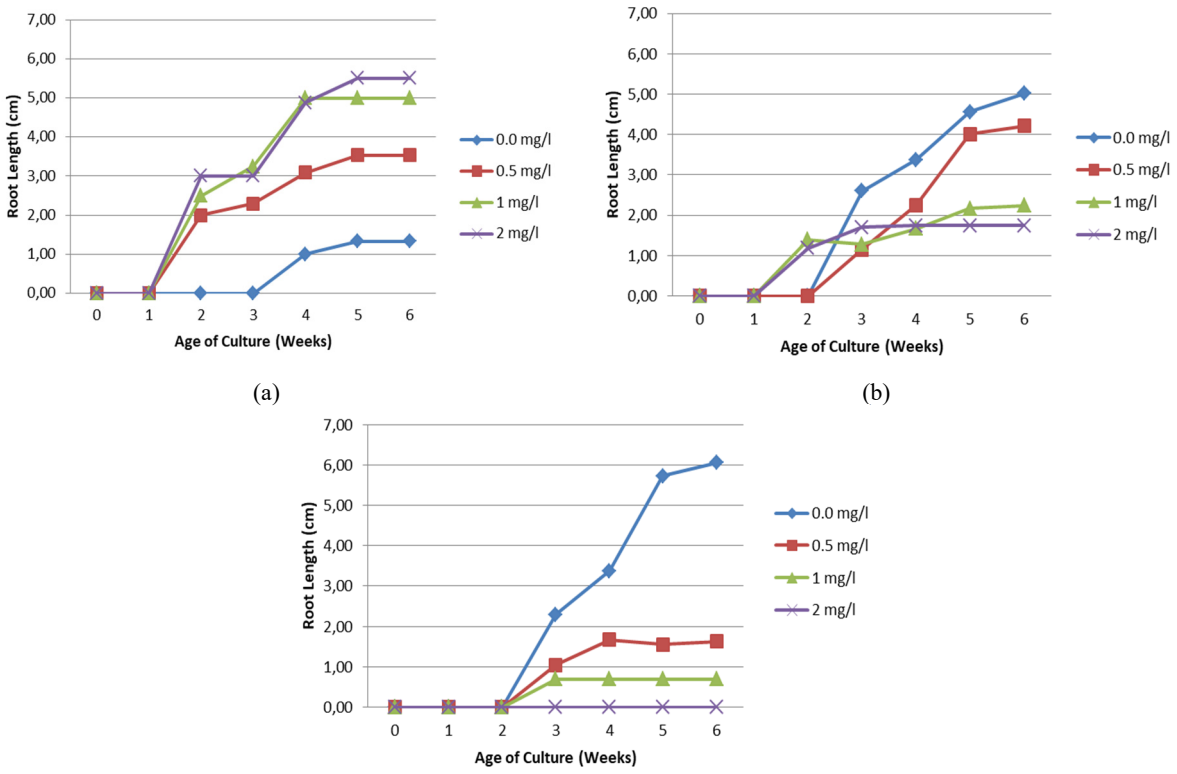

FIGURE 10. Root length of *M. oleifera* grown on DKW medium supplemented with 0.0; 0.5; 1.0 and 2.0 mg/l IAA (a), IBA (b), and NAA (c) refined for 0-6 weeks

TABLE 3. Variance analysis or ANOVA on shoot stature, shoot numbers, petiole numbers, root numbers and roots length of *M. oleifera* cultured on DKW media supplemented with IAA, IBA and NAA six weeks after culture

No	Variables	F Value & Significance			CV (%)
		Auxins	Concentrations	Auxins Vs Concentrations	
1.	Shoot Stature	26.64**	18.72**	4.31**	48.76
2.	Shoot Numbers	15.36**	7.09**	2.87*	44.62
3.	Petiole Numbers	41.62**	22.50**	1.94ts	47.03
4.	Root Numbers	41.13**	7.11**	11.91**	45.52
5.	Roots Length	10.93**	16.33**	12.94**	60.01

Note * : significance at α: 5%; **: very significance at α 1%; ns : not significance

Conversely, the medium containing 2.0 mg/l IAA acquired the most noteworthy roots at a month and a half. The most noteworthy roots length was created at the medium without IBA and NAA and contained 1.0 mg/l IAA (Table 4). However, in all the treatments, regardless of the different types of auxin and the different concentrations, the increasing hormone enhance the physiological response until reaching a saturation level. The increase of hormone concentration exceeding the "Saturation Level" could have an inhibitory physiological response. Higher hormone concentration does not impact the physiological response before exceeding the saturation point [27].

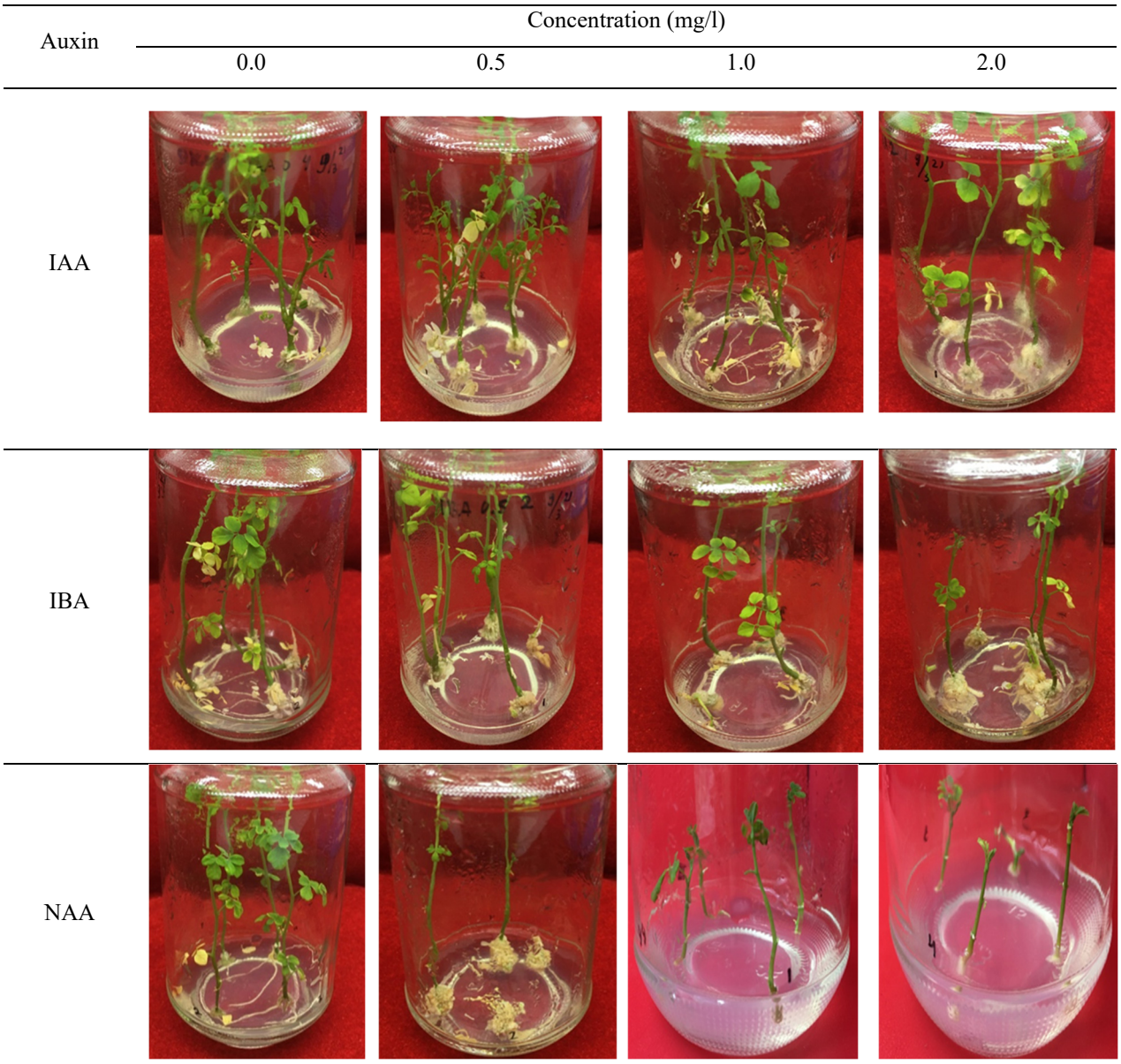

FIGURE 11. Shoot culture performance of *M. oleifera* on DKW medium supplemented with 0.0, 0,5, 1.0 and 2.0 mg/l IAA, IBA and NAA at six weeks after culture.

Figure 11 shows *M. oleifera* performance refined on DKW medium containing 0.0, 0,5, 1.0, and 2.0 mg/l IAA, IBA, and NAA at a month and a half after culture. In all control media (without adding IAA, IBA, or NAA), *M. oleifera* shoots had robust development with solid stems and green leaves, yet tiny roots were shaped (Fig. 11). Contrasts in the establishing capacities might be credited to the impact of every direction on the inward auxin transport framework inside the tissue. The auxin transport framework is polar, moving the direction into altered, vertical, or horizontal positions. At contrasting rates, auxin consumption from the cut surfaces relies upon the direction. Tissue changes in tissue's capacity to deliver adventitious organs have been connected to auxin consumption [29].

In the DKW medium with the expansion of 0.0 and 0.5 mg/l IAA and IBA, the shoot culture's exhibition was superior to the medium with IAA and IBA at 1.0 and 2.0 mg/l. Calli were formed in the medium containing 0.5, 1.0, and 2.0 mg/l IBA and NAA at the subordinate of the stems (Fig. 11). *M. oleifera* shoots developed on control medium (DKW medium only) looked vigorous and created wide leaves with long stems. Hindered shoots and a few

leaves were shaped on medium with 1.0 and 2.0 mg/l NAA. Some part of the stem enlarged and formed callus in the medium with 0.5 mg/l of NAA (Fig. 11).

TABLE 4. The average value of shoot stature, shoot numbers, petiole numbers, root numbers and root length of *M. oleifera* cultured on DKW medium containing 0.0, 0.5, 1.0 and 2.0 mg/l IAA, IBA and NAA 6 weeks after culture

Auxins	Concentration (mg/l)	Shoots Stature	Shoot Numbers	Petiole Numbers	Root Numbers	Root Length
IAA	0.0	9.68 ± 0.87^{b}	**2.42 ± 0.29^{a}**	**9.42 ± 0.83^{a}**	1.33 ± 0.14^{gh}	2.80 ± 0.36^{bc}
	0.5	8.94 ± 0.94^{bc}	2.17 ± 0.17^{ab}	8.67 ± 0.92^{ab}	3.58 ± 0.23^{cde}	2.36 ± 0.34^{c}
	1.0	8.24 ± 1.32^{bc}	1.33 ± 0.14^{cd}	5.42 ± 0.84^{cde}	5.00 ± 0.25^{ab}	**5.63 ± 0.88^{a}**
	2.0	10.03 ± 1.04^{b}	1.33 ± 0.19^{cd}	6.00 ± 0.69^{cd}	**5.50 ± 0.46^{a}**	3.25 ± 0.35^{bc}
IBA	0.0	**13.13 ± 0.64^{a}**	1.25 ± 0.18^{cd}	6.17 ± 0.27^{cd}	3.08 ± 0.48^{def}	5.26 ± 0.62^{a}
	0.5	8.23 ± 1.71^{bc}	1.75 ± 0.30^{bc}	5.83 ± 1.22^{cd}	3.50 ± 0.45^{cde}	4.16 ± 0.95^{ab}
	1.0	6.43 ± 1.20^{cd}	1.17 ± 0.11^{cd}	4.08 ± 0.65^{def}	4.17 ± 0.66^{bcd}	2.29 ± 0.37^{c}
	2.0	5.33 ± 0.84^{d}	1.25 ± 0.13^{cd}	3.58 ± 0.47^{ef}	4.67 ± 0.75^{abc}	1.75 ± 0.18^{cd}
NAA	0.0	9.69 ± 1.12b	1.42 ± 0.29^{cd}	6.75 ± 0.69^{bc}	2.37 ± 0.39^{efg}	5.59 ± 0.69^{a}
	0.5	4.85 ± 1.15^{d}	1.00 ± 0.00^{d}	2.75 ± 0.54^{fg}	2.00 ± 0.21^{fg}	1.63 ± 0.23^{cde}
	1.0	1.40 ± 0.07^{e}	1.00 ± 0.00^{d}	1.25 ± 0.13^{g}	2.00 ± 0.00^{fg}	0.70 ± 0.00^{de}
	2.0	1.10 ± 0.07^{e}	1.00 ± 0.00^{d}	1.00 ± 0.00^{g}	0.00 ± 0.00^{h}	0.00 ± 0.00^{e}

Note: The numbers followed by the same letter in the same column are not significantly different based on Duncan's multiple range test at α = 5%

CONCLUSION

Types and levels of cytokinins impacted shoot development of *M. oleifera* in vitro refined on DKW medium. Additionally, the sorts and levels of auxins likewise impacted its root development. Medium containing 1 mg/l Kinetin was excellent for shoot stature, shoot numbers and petiole numbers. Auxin IAA at 1.0 and 2.0 mg/l delivered the most significant root numbers and root length. Hence, the selection of PGR (plant growth regulators) was beneficial to find out the best medium composition for development and multiplication of *M. oleifera* shoots as well as for its root formation.

ACKNOWLEDGEMENTS

The authors might want to thank Risna Citra Andiani from Chemical Analyst Vocational School of Bogor, for her assistance in this research.

REFERENCES

1. F. Anwar, S. Latif, M. Ashraf, and A. Hassan. Phy. Res. **659**, 651–659 (2007).
2. S. Sahay, U. Yadav, S. Srinivasamurthy. Int. Jour. Food Scie. Nut. **2**, 31–37 (2017).
3. A.T., Oyeyinka, and S.A. Oyeyinka, Jour. Saudi Soc. Agric. Sci. **17**, 127–136 (2018).
4. T.G., Toyosi, O. Anthony, Obilanaa, B. Ayodeji, Oyenihib, and F. G. Rautenbach. South Afri. Jour. of Bot. **141**, 12-24 (2021).
5. B. Padayachee, and H. Baijnath. South Afric. Jour. of Bot. **129**, 304–316 (2020).
6. B.E.C., Ziani, W. Rached, K. Bachari, M.J. Alves, R.C. Calhelha, L. Barros, and I.C.F.R Ferreira. Jour. of Func. Foods **53**, 237–247 (2019).
7. A.A., Fernandesa, J. Bancessib, M.I Pinelaa, A. Diasa, R.C. Liberala, A. Calhelhaa, M. Ćirićc, L. Sokovićd, Catarinoc, I.C.F.R. Ferreiraa, and L. Barrosa. Food Che **341**, 128-229 (2021).
8. D. Sarkar. Plant Growth Reg. **62**, 117–125 (2010).
9. M.A. Mohamad, A. Yadollahi, A. Shojaeiyan, S. Shokri, and S.M. Ghojah. Jour. of Gen. Eng. and Biotech. **12**, 81–87 (2014).
10. K.R. Kondhare, A.B., Patil, and A.P. Giri. Plant Sci. **306**, 110-854 (2021).
11. M.F. Ashraf, M.A. Aziz, N. Kemat, and I. Ismail. Elec. Jour. of Biotech. **17**, 275–279 (2014).
12. P. Overvoordee, H. Fukaki, and T. Beeckman. Cold Spring Harbour Pers. in Bio. **2**, 15-37 (2010).

13. Rudiyanto, A. Purwito, D. Efendi and T.M.Ermayanti. *ICBEAU 2020*. (IOP Conf. Series: Earth and Environmental Science, 2020). pp. 741-756.
14. R.K. Saini, N.P. Shetty, P. Giridhar and G. A. Ravishankar. Biotech **2**, 187–192 (2012).
15. E.C. Marfori. Philipp Agric Sci. **93**, 454-457 (2010).
16. R.K. Radha, S.R. Shereena, K. Divya, P.N. Krishnan & S. Seeni. Int. Jour. of Bot. **7**, 90-96 (2011).
17. B.W. Hapsari, and T.M. Ermayanti. Jur. Bio. Ind. **16**, 25-37 (2020).
18. S.S. Bhojwani, and M.K. Razdan. (Elsevier 1996), pp. 356
19. Osugi and Sakakibara. *BMC Bio.* **13**, 102-116 (2015).
20. Rudiyanto, D. E. Rantau and T.M. Ermayanti. (Prosiding Seminar Nasional XXV "Kimia dalam Industri dan Lingkungan" Hotel Phoenix Yogyakarta, 17 Nopember 2016), pp. 103-111
21. M. Le Bris, Encyc. of Rose Sci. **2003**, 364-369 (2017).
22. T.M. Ermayanti, and E. Al Hafiizh. (Prosiding Seminar Nasional XVI "Kimia dalam Pembangunan "Hotel Phoenix Yogyakarta, 2013), pp. 733-741
23 B.W. Hapsari, Rudiyanto, and T.M. Ermayanti. (Prosiding Seminar Nasional Konservasi dan pemanfaatan tumbuhan dan Satwa Liar *"*Riset Sebagai Fondasi Konservasi dan Pemanfaatan Tumbuhan dan Satwa Liar*"* 2019), pp. 297-309
24. Azwin. Jur. Ilmiah Pert. **13**, 59-69 (2016).
25. T.R. Masekesa, E. Gasura, E. Ngadze, D. Icishahayo, G.T. Kujeke, F. Chidzwondo, and I. Robertson. South Afric. Jour. of Bot. **104**, 1–5 (2016).
26. P. Gupta, S. Sharma, and S. Saxena, World Acad. of Sci., Eng. and Tech. **4**, 12-25 (2010).
27. R. Zamir, N. Ali. S.T. Shah, T. Muhammad and S.A. Shah. PAK Jour. Bot. **39**, 2395-2398 (2007).
28. D.E. Rantau, Rudiyanto, D.R. Wulandari, B.W. Hapsari and T.M. Ermayanti. (Prosiding Seminar Nasional "Biodiversitas Untuk Kehidupan" ; Jakarta, 2018), pp. 236-249
29. S.M.A. Romman, A.K. Al-Hadid, and A.R. Arabiyyat. Jour. of Agric. Sci. **7**, 159-165 (2015).